Eur J Vasc Endovasc Surg **34**, 23–28 (2007)
doi:10.1016/j.ejvs.2007.02.008, available online at http://www.sciencedirect.com on ScienceDirect

Association of Increased Levels of Homocysteine and Peripheral Arterial Disease in a Japanese-Brazilian Population

L. Garofolo,[1*] N. Barros Jr.[2] F. Miranda Jr.[2] V. D'Almeida,[3] L.C. Cardien[3] and S.R. Ferreira[4]

[1]*Post-Graduation Course on Cardiac, Vascular and Thoracic Surgery, Surgery Department, Federal University of Sao Paulo, Sao Paulo, Brazil,* [2]*Vascular Surgery Division, Surgery Department, Federal University of Sao Paulo, Sao Paulo, Brazil,* [3]*Pediatrics Department, Federal University of Sao Paulo, Sao Paulo, Brazil, and* [4]*School of Public Health, University of Sao Paulo, Sao Paulo, Brazil*

Objectives. *Our aim was to evaluate the possible association between homocysteine levels and peripheral arterial disease (PAD) in a population-based study of Japanese-Brazilians.*
Materials and methods. *This cross-sectional study was derived from a population-based survey on the prevalence of diabetes and associated diseases conducted in Japanese-Brazilians. A total of 1330 male and female subjects aged ≥ 30 years were submitted to clinical examination and laboratory procedures including homocysteine measurement. The ankle-brachial index (ABI) was calculated; subjects with ABI values <0.9 were diagnosed with PAD. The evaluable population included 1008 subjects. Logistic regression was used taking PAD as the dependent variable.*
Results. *Mean age of the population was 56.5 years and overall prevalence of PAD was 20%. A worse cardiovascular profile was found in male patients, including significantly higher homocysteine levels (11.9 ± 1.8 vs. 9.1 ± 1.1 μmol/L, p < 0.001). Men with PAD had higher prevalence rates of hyperhomocysteinemia compared to women (22.7% vs 7.6%). Univariate analysis showed an odds ratio of hyperhomocysteinemia for PAD of 1.51 [1.02–2.25] in men and 1.69 [1.06–2.68] in women. After adjustment for other cardiovascular risk factors, higher levels of homocysteine were only significantly related to PAD in men.*
Conclusion. *In a Japanese-Brazilian population, elevated levels of homocysteine are associated with PAD in men. Prospective studies are necessary to confirm this finding.*

Keywords: Peripheral arterial disease; Homocysteine; Risk factor.

Introduction

The relationship between homocysteine and cardiovascular disorders has been extensively investigated, particularly in coronary and cerebrovascular conditions.[1–5] More recently, the association between homocysteine levels and peripheral arterial disease has also been highlighted.[6] In 1991, elevated levels of homocysteine were proposed as a novel independent risk factor for atherosclerotic diseases. In a case control study, subjects with coronary, cerebral and/or peripheral atherosclerosis showed elevated total homocysteine (tHcy) levels when compared to controls.[7] However, variable findings were subsequently reported in different populations. A meta-analysis based on 27 observational studies was carried out from 1976 to 1995 by Boushey *et al*. The odds ratio (OR) for CAD of a 5 umol/L tHcy increment is 1.6 (95% confidence interval [CI], 1.4 to 1.7) for men and 1.8 (95% CI, 1.3 to 1.9) for women. A total of 10% of the population's CAD risk appears attributable to total homocysteine. The authors concluded that a 5 μmol/L total homocysteine increment elevates CAD risk by as much as cholesterol increases of 0.5 μmol/L (20 mg/dL).[4] Similarly, a prospective study reported a relative risk increment of 1.41 for every 4 μmol/L increase in homocysteine concentration.[8]

Our group has conducted a population-based study with Japanese immigrants and their descendants in Brazil, since they have one of the highest prevalence rates of glucose metabolism disturbances worldwide.[9] High prevalence rates of type 2 diabetes, hypertension and dyslipidemia in Japanese immigrants living in the Americas[10,11] suggest that the exposure to a different lifestyle exacerbated an inherent tendency to accumulate body fat and increase cardiovascular risk factors. More recently, 57% of Japanese-Brazilians were shown

*Corresponding author. L. Garofolo, Rua Manoel da Nóbrega, 964 apto. 28, Jardim Paulista, CEP 04001-003, São Paulo, SP, Brazil.
E-mail address: lgarofolo@ig.com.br

1078–5884/000023 + 06 $32.00/0

to have metabolic syndrome.[12] Therefore, this population represented an opportunity to investigate the possible relationship between newly identified risk factors, such as circulating levels of homocysteine, and atherosclerotic cardiovascular diseases. Data collected in 2000 from the Japanese-Brazilian population aged ≥30 years included Doppler of the extremities and a number of laboratory tests.

Our aim was to investigate whether elevated levels of homocysteine were independently associated with peripheral arterial disease in this population of Japanese-Brazilians with high cardiovascular risk.

Methods

A survey was conducted in a population of first (Japan-born) and second-generation Japanese-Brazilians living in Bauru, São Paulo, Brazil, to estimate the prevalence of diabetes and associated diseases. The data used subsequently in this analysis belong to the second phase of the survey, conducted in 2000. All the patients, male and female, ≥30 years of age were invited to participate. Details on the selection and recruitment of the sample population have been previously described.[13] The Institutional Ethics Committee approved the study, and informed consent was obtained from all the subjects. A total of 1330 subjects were interviewed at home by trained interviewers, using standardized health and nutritional status questionnaires, and were scheduled for clinical and laboratory procedures. Data on smoking status (non-smoker, current or previous smokers, i.e. subjects who had smoked 5 or more cigarettes a day for at least 12 months) and past medical history were recorded.

The clinical examination included anthropometric measurements. Body mass index, determined by the weight (kilograms) divided by the square of the height (meters); waist circumference was measured at the umbilicus level. Blood pressure was taken after 5 minutes rest in the sitting position, with an automatic device (Omron model HEM-712C, Omron Health Care, Inc, USA). The mean of the last 2 measurements was used to express systolic and diastolic blood pressure values. Fasting blood samples were taken for several tests. Non-diabetic and self-reported diabetic subjects with fasting capillary glucose <200 mg/dL, screened with glucose-oxidase strips, were submitted to a 75-gram oral glucose tolerance test.

Conventional risk factors

Generalized and central obesity was defined according to WHO criteria for Asian subjects.[14] The cutoffs of waist circumference for Asian populations were 90 cm for men and 80 cm for women. Hypertension was defined by systolic/diastolic blood pressure ≥ 140/90 mmHg or use of antihypertensive medications. The diagnosis of dyslipidemia was based on the Third Report of the National Cholesterol Education Program − ATP III.[15] Hyperuricemia was defined as uric acid values above 7 mg/dL for men and 6 mg/dL for women.[16] Glucose tolerance status was based on 1999 WHO criteria.[17]

Peripheral arterial disease diagnosis

The transcutaneous blood velocity detector used was the Imbracios-8MHz Doppler. Initially, brachial occlusion pressure was recorded in the supine position with the Doppler probe placed over the brachial artery at the elbow. The measurement was taken in both arms and the highest value was retained. Then, ankle occlusion pressure was recorded with the pencil probe placed on the anterior and posterior tibial arteries. The ankle-brachial index was determined in both extremities. An index below 0.90 indicated flow-limiting stenosis in the examined leg[18] and established the diagnosis of PAD. Subjects with chronic renal failure (serum creatinine > 1.6 mg/dL or clearance of 60 mL/min),[19] or without complete clinical examination, laboratory profile or ankle-brachial index data were excluded. Therefore, data from 1008 participants (459 men and 549 women) were analyzed.

Analytical methods

Plasma glucose, creatinine and uric acid concentration were determined by routine methods. Cholesterol lipoprotein fractions and triglycerides were measured by enzymatic methods in automatic analyzers. Homocysteine was determined by high performance liquid chromatography, as described by Pfeiffer *et al*.[20] (normal range: 5−15 μmol/L) and detected by a fluorimetric method. Plasma homocysteine levels exceeding 15 μmol/L were considered to be elevated.[21] Sera were stored at −70 °C.

Statistical analysis

Data were reported as mean and standard deviation for numerical variables or number and percent for categorical variables. Unpaired Student's *t* test was used to compare means of clinical and laboratory data between subgroups stratified by gender. Chi-square test was used for comparisons of frequencies. Non-conditional logistic regression analysis was applied,

taking PAD as the dependent variable and hyperhomocysteinemia as the independent variable of main interest, adjusted for a number of categorical variables (conventional risk factors). Those variables with a p value ≤ 0.20 in the crude analysis were selected for the multivariate analysis. Distinct models were built for each gender. Results were expressed as odds ratios and 95% confidence intervals. The initial model for men included age (continuous variable), smoking habit (non-smoker, former or current smoker), glucose tolerance status and systemic hypertension, in addition to hyperhomocysteinemia; for women, dyslipidemia and hyperuricemia were also included. The level of significance was set at alpha = 0.05. Data analyses were performed using STATA 7.0.

Results

The mean age of the 1008 Japanese-Brazilian subjects studied (46% male) was 56.5 years. Sixty-two percent of the population was in the 50−69 years age group; both genders had similar age distribution (Table 1). The mean values of body mass index, waist circumference, blood pressure, fasting and 2-hour plasma glucose, HDL-cholesterol, triglycerides, uric acid and homocysteine were significantly higher in male subjects.

Table 2 shows the prevalence rates of conventional risk factors and of hyperhomocysteinemia in Japanese-Brazilian men and women.

The prevalence of PAD was estimated at 20.4% (95% CI [18.0%;23.1%]) for the total population and no difference was found between genders (19.1% *vs*. 20.7%, $p = 0.529$). The odds ratio for PAD per cardiovascular risk factor is presented in Table 3.

Univariate analysis showed higher OR for PAD in former or active smoker versus non-smoker male subjects ($p < 0.05$) (Table 3). In female subjects aged ≥ 60 years, the presence of hypertension, diabetes, hypercholesterolemia, and hypertriglyceridemia was significantly associated with increased risk of PAD. Hyperhomocysteinemia also increased the risk of PAD in both genders (OR 1.51 and 1.69, respectively, for men and women, $p < 0.05$).

The logistic regression model for males showed that PAD was independently associated with age, smoking habit (former and current) and hyperhomocysteinemia (Table 4). The risk of developing

Table 2. Prevalence rates of peripheral arterial disease risk factors (%) by gender

	Men	Women	Total	*P*
Age ≥ 60 years	42.0	41.5	41.8	0.868
Smoking habit				
Non-smokers	46.3	87.7	68.8	<0.001
Former smokers	34.5	4.5	18.2	
Current smokers	19.2	7.8	13.0	
Nutritional status				
Normal	48.2	61	55.3	<0.001
Overweight	41.4	29.3	34.7	
Obesity	10.4	9.7	10.0	
Central obesity	42.5	46.1	44.4	0.252
Hypertension	45.2	41.2	43.0	0.219
Glucose tolerance				
Normal	9.5	24.8	17.9	<0.001
Abnormal fasting plasma glucose	25.5	19.6	22.3	
Impaired glucose tolerance	26.4	25.9	26.1	
Diabetes	38.6	29.7	33.7	
Total cholesterol > 200 mg/dL	58.4	61.2	59.9	0.364
LDL-cholesterol > 130 mg/dL	42.5	49.7	46.4	0.022
Low HDL-cholesterol*	6.1	24.7	16.3	<0.001
Triglyceride >150 mg/dL	74.1	59.9	66.4	<0.001
Hyperuricemia**	45.1	24.9	34.1	<0.001
Hyperhomocysteinemia	22.7	7.6	14.5	<0.001

* Reference value: men, <35 mg/dL; women <45 mg/dL.
** Reference value: men, 7 mg/dl; women, 6 mg/dl.

Table 1. Main characteristics of the Japanese-Brazilian population by gender

	Male $N = 459$	Female $N = 549$	Total $N = 1008$	*p*
Age (years)	56.7 (13.1)	56.4 (12.3)	56.5 (12.7)	0.671
Body mass index (kg/m^2)	25.4 (3.8)	24.5 (3.9)	24.9 (3.9)	<0.001
Waist circumference (cm)	88.5 (9.8)	79.8 (9.6)	83.8 (10.6)	<0.001
Systolic blood pressure (mmHg)	135.3 (23.2)	130.2 (25.0)	132.5 (24.3)	<0.001
Diastolic blood pressure (mmHg)	81.3 (13.2)	76.9 (13.2)	78.9 (13.4)	<0.001
Fasting plasma glucose (mg/dL)	127.0 (31.0)	120.5 (31.6)	123.5 (31.5)	0.001
2-hour plasma glucose (mg/dL)	167.1 (85.3)	154.2 (72.6)	160.1 (78.9)	0.010
Total cholesterol (mg/dL)	211.9 (42.3)	212.9 (41.0)	212.5 (41.6)	0.699
LDL-cholesterol (mg/dL)	127.3 (37.0)	129.4 (36.5)	128.5 (36.7)	0.375
HDL-cholesterol (mg/dL)	49.1 (12.1)	51.5 (10.0)	50.4 (11.0)	0.001
Triglycerides (mg/dL)	268.9 (223.5)	195.8 (132.6)	229.2 (189.2)	<0.001
Uric acid (mg/dL)	7.0 (1.8)	5.3 (1.3)	6.1 (1.8)	<0.001
Homocysteine (μmol/L)	11.9 (1.8)	9.1 (1.1)	10.3 (1.0)	<0.001

Data expressed as means and standard deviation (in parenthesis).

Table 3. Odds ratios of risk factors for peripheral arterial disease in Japanese-Brazilian subjects, by gender

	Men			Women		
	OR	95% CI	*p*	OR	95% CI	*p*
Age ≥ 60 years	1.38	0.94–2.00	0.093	**1.68**	**1.21–2.33**	**0.002**
Smoking						
Former smokers	**1.80**	**1.13–2.86**	**0.012**	0.93	0.42–2.08	0.862
Current smokers	**2.49**	**1.54–4.01**	**0.001**	0.54	0.23–1.26	0.127
Waist circumference	0.77	0.52–1.15	0.196	1.02	0.73–1.41	0.922
Hypertension	1.33	0.91–1.93	0.138	**1.95**	**1.40–2.71**	**<0.001**
Glucose tolerance status						
Abnormal fasting plasma glucose	0.98	0.47–2.06	0.972	1.13	0.64–2.00	0.652
Impaired glucose tolerance	0.90	0.43–1.91	0.082	1.50	0.91–2.47	0.100
Diabetes	1.21	0.61–2.40	0.576	**1.62**	**1.01–2.61**	**0.039**
Total cholesterol > 200 mg/dL	1.19	0.80–1.75	0.384	**1.56**	**1.08–2.24**	**0.015**
LDL-cholesterol > 130 mg/dL	0.94	0.64–1.37	0.740	1.34	0.96–1.87	0.080
Low HDL-cholesterol*	0.54	0.18–1.61	0.241	1.08	0.75–1.57	0.668
Triglyceride >150 mg/dL	0.99	0.58–1.67	0.960	**1.85**	**1.18–2.89**	**0.007**
Hyperuricemia**	1.22	0.84–1.77	0.304	1.33	0.94–1.89	0.111
Hyperhomocysteinemia	**1.51**	**1.02–2.25**	**0.046**	**1.69**	**1.06–2.68**	**0.037**

* Reference value: men, <35 mg/dL; women <45 mg/d.
** Reference value: men, 7 mg/dl; women, 6 mg/dl.

PAD increases 2% with each year of age ($p = 0.02$); each increase of 1 μmol/L in the homocysteine level was associated with a 3% increase in the risk of developing peripheral arterial disease ($p = 0.02$).

In female subjects, the logistic regression analysis also detected an independent association between age ($p = 0.04$) and hypertension ($p = 0.01$) with PAD (Table 5).

Discussion

A high prevalence of PAD (20.4%) was detected in this population-based study of Japanese-Brazilian subjects. Considering that this population has one of the highest prevalence rates of diabetes worldwide[12] as well as of other conventional cardiovascular risk factors,[8,22,23] they are also expected to have a high prevalence of atherosclerotic disease.

The ankle-brachial index has been widely used as the diagnostic tool for PAD.[18,24–27] In the Limburg's study, using the same methodology, Stoffers *et al*.[28] found a lower prevalence of PAD (6.1%) than in our study, and could not detect differences in rates between genders. Our data agree with those from the Rotterdam study, where prevalence rates were approximately 17% in male and 20% in female subjects above age 55.[26]

More recent data from the *National Health and Nutrition Examination Survey*[29] found PAD was present in 4.3% of the North American population above 40 years and in 14.5% above 70 years. Murabito *et al*.[30] found similar data analyzing 1554 men and 1759 women included in the Framingham study. Using the ankle-brachial index for diagnosing PAD, their prevalence rate was 3.9% in men and 3.3% in women. Despite these lower rates, again there was no difference between genders. The high incidence of conventional risk factors in both genders might contribute to similar rates of PAD in Japanese-Brazilian men and women.

Using multivariate analysis, we found a number of risk factors associated with PAD for each gender. Age was associated with PAD in men and women. Smoking and hyperhomocysteinemia were only associated with PAD in men. In fact, the prevalence of smoking among women was low. Even with the low prevalence of smoking among women, we found a similar prevalence of PAD in both sexes; therefore, the high prevalence of PAD in women may be

Table 4. Logistic regression model for peripheral arterial disease in Japanese-Brazilian men

	Odds ratio	95% CI	*P* value
Age	1.02	1.00–1.04	0.02
Former smokers	1.82	1.03–3.21	0.04
Current smokers	3.22	1.76–6.04	0.01
Hyperhomocysteinemia	1.03	1.00–1.06	0.02

CI, confidence interval.

Table 5. Logistic regression model for peripheral arterial disease in Japanese-Brazilian women

	Odds ratio	95% CI	*P* value
Age	1.57	1.02–2.44	0.04
Hypertension	2.05	1.32–3.18	0.01

CI, confidence interval.

influenced by hyperhomocysteinemia. Univariate analysis of risk factors in women showed an odds ratio of hyperhomocysteinemia for PAD of 1.69 (1.06−2.68).

Homocysteine concentration, as well as the prevalence of hyperhomocysteinemia, were lower in Japanese-Brazilian women than in men. These findings are similar to those obtained in a smaller study, including 32 women and 29 men with PAD.[31] The investigators found significantly higher levels of homocysteine among men (18.3 μmol/L) as compared to women (15.3 μmol/L). No association between PAD and hyperhomocysteinemia in the female subjects was also demonstrated in another investigation.[32]

Loncar *et al*.[33] compared homocysteine levels of 40 subjects with PAD and 40 healthy volunteers, and found higher mean values in affected subjects (14.8 μmol/L vs 11.3 μmol/L). Differences in homocysteine concentrations between genders were observed in the control group, with significantly higher values in healthy men than in healthy women (12.05 μmol/L vs 9.63 μmol/L). Such influence of gender on homocysteine levels was first described by Cacan *et al*.[34] in a healthy study population; mean values of 9.7 μmol/L in men were shown to be significantly higher than in women (7.6 μmol/L). A plausible explanation for this difference was proposed by Dierkes *et al*.[35] who suggested that high levels of estradiol and low percentage of lean body mass could contribute to lower homocysteine levels in female subjects.

We defined hyperhomocysteinemia as plasma homocysteine levels above 15 μmol/L. We did not consider the influence of gender on homocysteine levels, which may have interfered with the low prevalence of female hyperhomocysteinemia. In our cross-sectional study, the prevalence of PAD in Japanese-Brazilian men increased 3% for each 1 μmol/L elevation in homocysteine concentration. These data are similar to those reported in the *Homocysteine and Progression of Atherosclerosis Study*.[36] This prospective study concluded that an elevation of 1 μmol/L in serum homocysteine increased by 3.6% the risk of death from PAD. A recent review[37] confirmed that the odds ratio for PAD in subjects with hyperhomocysteinemia varies considerably, ranging from 2.0 to 11.0.

Clarke *et al*.[7] were the first to report that hyperhomocysteinemia could be an independent risk factor for atherosclerosis. In subjects with hyperhomocysteinemia, they found different odds ratios, depending on the arterial territory studied: 1.52 for extracranial carotid-artery, 1.40 for coronary artery and 1.13 for peripheral arteries. The hypothesis that hyperhomocysteinemia could be a novel atherosclerotic risk factor was contested by Valentine *et al*.,[38] who did not find any difference in homocysteine values comparing young men with and without PAD.

The Nutrition Examination Survey (NHANES),[39] a cross-sectional study, evaluated the roles of traditional and non traditional risk factors, including lead and cadmium exposure, in confounding the association between homocysteine and reduced ankle-brachial index. The findings indicate that previous studies may have overestimated the association between homocysteine and PAD. Adjustment for blood lead and cadmium and for smoking completely eliminated the association of homocysteine with PAD. On the contrary, our study found independent associations of homocysteine and smoking with PAD in men.

In conclusion, our data supports an independent association of PAD and hyperhomocysteinemia in Japanese-Brazilian male subjects. The study design does not allow a causal relationship to be assigned. To find out if an elevated homocysteine level represents an additional risk factor for this population, prospective studies are required, perhaps considering different normal ranges for men and women.

References

1 Nygard O, Nordrehaug JE, Refsum H. Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Engl J Med* 1997;**337**:230−236.
2 Nygard O, Vollset SE, Refsum H. Total homocysteine and cardiovascular disease. *J Intern Med* 1999;**246**:425−454.
3 Graham IM, Daly LE, Refsum HM. Plasma homocysteine as a risk factor for vascular disease: the European Concerted Action Project. *JAMA* 1997;**277**:1775−1781.
4 Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. *JAMA* 1995;**274**(13):1049−1057.
5 Danesh J, Lewington S. Plasma homocysteine and coronary heart disease: systematic review of published epidemiological studies. *J Cardiovasc Risk* 1998;**5**:229−232.
6 McCully KS. Vascular pathology of homocysteinemia: implications for pathogenesis of atherosclerosis. *Am J Pathol* 1969;**56**: 111−128.
7 Clarke R, Daly L, Robinson K. Hyperhomocysteinemia: an independent risk factor for vascular disease. *N Engl J Med* 1991;**324**: 1149−1155.
8 Arnesen E, Refsum H, Bonaa KH, Ueland PM, Forder OH, Nordrehaug JE. Serum total homocysteine and coronary artery disease. *Int J Epidemiol* 1995;**24**:704−709.
9 Gimeno SGA, Ferreira SRG, Cardoso MA, Franco LJ, Iunes M, Japanese-Brazilian Study Group, 2000a. Weight gain in adulthood and risk of developing glucose tolerance disturbance: a study of Japanese-Brazilian Population. *J Epidemiol* 2002;**10**: 103−110.
10 Fujimoto WY, Leonetti DL, Kinyoun JL, Newell-Morris L, Shuman WP, Storov WC *et al*. Prevalence of diabetes mellitus and impaired glucose tolerance among second generation Japanese American men. *Diabetes* 1987;**36**:721−729.
11 Ferreira SRG, Iunes M, Franco LJ, Iochida LC, Hirai A, Vivolo MA. Disturbances of glucose and lipid metabolism in first and second generation Japanese-Brazilians. *Diabetes Res Clin Pract* 1996;**34**(Suppl):S59−S63.

12 Rosenbaum P, Gimeno SGA, Sanudo A, Franco LJ, Ferreira SRG. Analysis of criteria for metabolic syndrome in a population-based study. *Diabetes Obes Metab* 2005;**7**(4):352–359.
13 Lerario Daniel DG, Gimeno Suely G, Franco Laércio J, Iunes Magid, Ferreira Sandra RG. Weight excess and abdominal fat in the metabolic syndrome among Japanese-Brazilians Rev. *Saúde Pública* 2002;**36**(1).
14 Bassett J, Inoue S, Zimmet P, Caterson I, Chunming C, Ikeda Y *et al*. The Asia-Pacific Perspective: Redefining Obesity and Treatment. Health Communications Australia; 2000.
15 Grundy MS, Becker D, Clark LT. Executive summary of the report of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adults treatment panel III). *Circulation* 2002;**106**:3143–3421.
16 Becker Michael A, Ralph Schumacher Jr H, Wortmann Robert L, MacDonald Patricia A, Eustace Denise, Palo William A *et al*. Febuxostat compared with allopurinol in patients with hyperuricemia and gout. *N Engl J Med* Dec 8, 2005;**353**(23):2450–2461. Boston.
17 Modan M, Harris MI, Halkin H. Evaluation of WHO and NDDG criteria for impaired glucose tolerance. Results from two national samples. *Diabetes* 1989;**38**(12):1630–1635.
18 Yao JST. Haemodinamic studies in peripheral arterial disease. *Br J Surg* 1970;**57**:762–766.
19 Kon V, Badr KF. Biological actions and pathophysiologic significance of the endothelin in the kidney. *Kidney Int* 1991;**40**:1–12.
20 Pfeiffer CM, Huff DL, Gunter EW. Rapid and accurate HPLC assay for plasma total homocysteine and cysteine in a clinical laboratory setting. *Clin Chem* 1999;**45**:290–292.
21 Refsum H, Ueland PM, Nygard O, Voilset SE. Homocysteine and cardiovascular disease. *Annu Rev Med* 1988;**49**:31–62.
22 Freire RD. Estado Nutricional de Nipo-Brasileiros de Alto Risco para Síndrome Metabólica: Análise por Sexo e Geração. Dissertação de Mestrado, São Paulo: Escola Paulista de Medicina, Universidade Federal de São Paulo; 2002.
23 Gimeno SGA, Ferreira SRG, Franco LJ, Iunes M, Katsumi O, Japanese-Brazilian Diabetes Study Group. Incremento na mortalidade associada à presença de diabetes mellitus em nipo-brasileiros. *Rev Saúde Pública* 1998;**32**:118–124.
24 Ouriel K. Peripheral arterial Disease. *Lancet* 2001;**358**:1257–1264.
25 Hiatt WR, Hoag S, Hamman RF. Effect of diagnostic criteria on the prevalence of peripheral arterial disease. The San Luis Valley Diabetes Study. *Circulation* 1995;**91**:1472–1479.
26 Meijer WT, Hoes AW, Rutgers D, Bots ML, Hofman A, Grobbee DE. Peripheral arterial disease in the elderly: the Rotterdam Study. *Arterioscler Thromb Vasc Biol* 1998;**18**:185–192.
27 Beach KW, Bedford GR, Bergelin RO, Martin DC, Vanderberghe N, Zaccardi M *et al*. Progression of lower extremity arterial occlusive disease in Type II diabetes Mellitus. *Diabetes Care* 1988;**11**(6):464–472.
28 Stoffers HE, Kaiser V, Knottnerus JA. Prevalence in general practice. In: Fowkes FGR, ed. *Epidemiology of peripheral vascular disease*. London: Springer-Verlag; 1991:109–115.
29 Selvin E, Erlinger TP. Prevalence of and risk factors for peripheral arterial disease in the United States. Results from the National Health and Nutrition Examination Survey, 1999–2000. *Circulation* 2004;**110**:738–743.
30 Murabito JM, Evans JC, Nieto K, Larson M, Levy D, Wilson PWF. Prevalence and clinical correlates of peripheral arterial disease in the Framingham Offspring Study. *Am Heart J* 2002;**143**:961–965.
31 Loehrer FMT, Tschöpl M, Angst CP, Litynski P, Kurt Jäger, Fowler B *et al*. Disturbed ratio of erythrocyte and plasma S-adenosylmethionine/S-adenosylhomocysteine in peripheral arterial occlusive disease. *Atherosclerosis* 2001;**154**:147–154.
32 Bloemenkamp DGM, Van den Bosch MAAJ, Mali WPTM, Tanis BC, Rosendaal FR, Kermmeren JM *et al*. Novel risk factors for peripheral arterial disease in young women. *Am J Med* 2002;**113**:462–467.
33 Loncar R, Hrboka V, Tabakovic-Loncar V, Ostojic Z, Deussen A. Screening of plasma homocysteine in peripheral arterial disease. *Ann Med* 2001;**33**:48–54.
34 Cacan SL, Xhignesse M, Piolot A, Selhub J, Davignon J, Genest JJ. Plasma total homocysteine in health subjects: sex-specifc relation with biological traits. *Am J Clin Nutr* 1996;**64**:587–593.
35 Dierkes J, Jeckel A, Ambrosch A, Westphal S, Luley C, Boeing H. Factors explaining the difference of total homocysteine between men and women in the european investigation into cancer and nutrition potsdam study. *Metabolism* 2001; **50**(6):640–645.
36 Taylor LM, Moneta GL, Sexton GJ, Schuff RA, Porter JM. Prospective blinded study of the relationship between plasma homocysteine and progression of symptomatic peripheral arterial disease. *J Vasc Surg* 1998;**29**(1):8–21.
37 Kuan YM, Dear AE, Grigg MJ. Homocysteine: an aetiological contributor to peripheral vascular arterial disease. *ANZ J Surg* 2002;**72**:668–671.
38 Valentine RJ, Jackson MR, Modrall JG, McIntyre KE, Clagett GP. The progressive nature of peripheral arterial disease in young adults: a prospective analysis of with men referred to a vascular surgery service. *J Vasc Surg* 1999;**30**(3):436–445.
39 Guallar E, Silbergeld EK, Navas-Acien A, Malhotra S, Astor BC, Sharrett AR *et al*. Confounding of the relation between homocysteine and peripheral arterial disease by lead, cadmium, and renal function. *Am J Epidemiol* 2006;**163**: 700–708.

Accepted 13 February 2007
Available online 7 May 2007